U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-294 909

Effects of Some Trace Elements on the Blood of Kuwait Mullets 'Liza macrolepis' (Smith)

Kuwait Inst for Scientific Research Marine Pollution Programme

Prepared for

Environmental Research Lab -Duluth, MN

1978

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. PB 294909
EPA-600/J-78-095	JOURNAL ARTICLE	

4. TITLE AND SUBTITLE	5. REPORT DATE
Effects of Some Trace Elements on the Blood of Kuwait Mullets Liza macrolepis (Smith)	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
M. M. Helmy, A. E. Lemke, P. G. Jacob, and B. L. Oostdam	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
Marine Pollution Programme Environmental and Earth Science Division Kuwait Institute for Scientific Research Kuwait	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
U. S. Environmental Protection Agency Environmental Research Lab.-Duluth Duluth, MN 55804	14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES REFERENCE: Helmy, M. M., A. E. Lemke, P. G. Jacob and B. L. Oostdam. 1978. Effects of Some Trace Elements on the Blood of Kuwait Mullets, Liza macrolepis (Smith). J. Exp. Mar. Biol. Ecol. 34: 151-161.

16. ABSTRACT

Haemopathological changes attributed to heavy metal poisoning observed in blood smears of Liza macrolepis (Smith) taken after exposures of 96 h to graded doses (mg/l) of copper (0.11-1.80), lead (1.15-18.36), and mercury (0.04-0.59), in a flow-through marine bioassay system. In general, changes in leucocytic profile appear to be correlated with pathological changes caused by increasing copper and mercury concentrations. By contrast, blood samples of mullets exposed to lead, showed significant polychromasia and +1 anisocytosis regardless of concentrations. The RBC count, haemoglobin content, and haematocrit percentages were less valuable in diagnosis of copper and mercury effects.

These manifestations of poisoning by trace elements bear a resemblance to the pathological changes that have been shown clinically and experimentally in mammals. Consequently, blood measurements on marine organisms may be diagnostic of undesirably high levels of copper and mercury, and so may constitute useful indicators of marine pollution.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Heavy metals Haemoglobin Haemeotocrit Leucocyte Copper Mercury Lead	Blood parameters Mullet Marine organisms	06/F 08/A

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	13
	20. SECURITY CLASS (This page) UNCLASSIFIED	22. PRICE RA02 MF A01

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA-600/J-78-095
JOURNAL ARTICLE

J. exp. mar. Biol. Ecol., 1978, Vol. 34, pp. 151–161
Elsevier/North-Holland Biomedical Press

EFFECTS OF SOME TRACE ELEMENTS ON THE BLOOD OF KUWAIT MULLETS, *LIZA MACROLEPIS* (Smith)

M. M. HELMY, A. E. LEMKE, P. G. JACOB and B. L. OOSTDAM
Marine Pollution Programme, Environmental and Earth Science Division, Kuwait Institute for Scientific Research, Kuwait

Abstract: Haemopathological changes attributed to heavy metal poisoning were observed in blood smears of *Liza macrolepis* (Smith) taken after exposures of 96 h to graded doses (mg l) of copper (0.11–1.80), lead (1.15–18.36), and mercury (0.04–0.59), in a flow-through marine bioassay system. In general, changes in leucocytic profile appear to be correlated with pathological changes caused by increasing copper and mercury concentrations. By contrast, blood samples of mullets exposed to lead, showed significant polychromasia and + 1 anisocytosis regardless of concentrations. The RBC count, haemoglobin content, and haematocrit percentages were less valuable in diagnosis of copper and mercury effects.

These manifestations of poisoning by trace elements bear a resemblance to the pathological changes that have been shown clinically and experimentally in mammals. Consequently, blood measurements on marine organisms may be diagnostic of undesirably high levels of copper and mercury, and so may constitute useful indicators of marine pollution.

INTRODUCTION

Clinical chemical analyses used in mammalian studies are highly developed and reliable, but only modest application of the principles and techniques has been extended to studies of aquatic species. Evidence is, however, accumulating that valid and useful analytical relations may be drawn between biochemical-physiological factors and the pathology of marine organisms.

The importance of reliable pre-mortal signs of toxicity and the need for biological systems for monitoring effluents are well recognized in the current literature related to water quality. Although some characteristics of fish blood have been monitored after exposure to pollutants, a review of the literature indicates lack of the use of fish blood morphology as a biological index of water quality. The purpose of the present study was, by a systematic haematological examination, to evaluate the use of pathological sequels of blood dyscrasia induced in Kuwait mullets, *Liza macrolepis* (Smith) *viz.*, fingerlings (Kuronuma & Abe, 1972), as short-term indicators to predict the effect of chronic exposures to copper, lead, and mercury. These three trace elements were selected because they are reported to be the prevalent cause of pollution offshore in Kuwait (Occupational Health and Industrial Pollution Control Section, 1976).

The pathological effects of the above trace elements in humans and in experimental

animals and fish are well documented: copper (Sprague, 1964; Hubschman, 1967; Mount & Stephan, 1967); mercury (Aaronson & Spiro, 1973; Miller & Clarkson, 1973); and lead (Hammond, 1969; Hardy *et al.*, 1973). Nevertheless, the precise toxicological modalities of the metals to various species need to be elucidated.

A few haematological characteristics have been investigated in fishes under known conditions of trace element exposures, employing techniques adapted from mammalian haematology. For instance, in carp (*Cyprinus carpio*) exposed to cobalt, Frolova (1960) found that the erythrocyte levels and haemoglobin concentrations increased, whereas the number of leucocytes declined. The microhaematocrit, haemoglobin concentrations, and red cell counts may be useful in the evaluation of stress in fishes, but reliance upon these measurements alone may not always reveal a haematological response (Houston & de Wilde, 1968). A broader knowledge of the pathological effects on circulating blood elements caused by water pollutants could provide the framework for a classification similar to those routinely used in mammalian toxicology.

Materials and Methods

Because of their widespread distribution, easy availability and economic importance, Kuwait mullets, *Liza macrolepis* (Smith), were used. Yearly fingerlings were collected from natural populations in the vicinity of the Marine Biology and Fisheries Division of the Kuwait Institute for Scientific Research, Salmiyah, during the period of April to June, 1977. All the fish used were apparently healthy and as uniform in size and age as possible. Only medium sized specimens weighing between 2.8 and 4.1 g (mean 3.3 g) were used, with average standard length (tip of snout to end of caudal peduncle) of 6.6 cm (range 6.0–7.5 cm).

Following collection, the fish were acclimated in a 340 l tank for one week prior to toxicant exposure, thus minimizing the effects of stress during the collection and transport. They were fed daily between 8 and 9 a.m. with dry prepared mullet food (Glenco Mills, Inc., Glenco, Minnesota), until 24 h before testing. A minimum of 20 fish were used for the control and for each concentration of metal to be tested. These organisms were equally divided as replicates between four tanks of 40 l each to avoid crowding.

The short term exposures (96 h) to copper, lead and mercury were carried out in the serial diluter described by Mount & Brungs (1967) using relatively pure sea water pumped off Salmiyah. This continuous flow-through system delivered constant metal concentrations as well as the control sea water, at a flow rate of 200 ml/min for each concentration, into glass tanks 31 × 31 × 62 cm with a 21 cm high standpipe drain at one end. Continuous flow-through systems offer the advantages of replenishment of dissolved oxygen and nutrients vital to test organisms, removal of volatile and other unstable components of the toxicant and the metabolic wastes

of the test organisms, and also more accurate estimates of absolute toxicity (Burdick, 1966; Lichatowich *et al.*, 1973).

Dissolved oxygen was measured in two tanks each day, and its concentration remained close to saturation. Other water properties of the system were monitored in one tank daily. The daily light lasted for 12 h, starting at 06.45. A reagent grade, cupric sulphate ($CuSO_4 5H_2O$) stock solution 25.5 g/l, was acidified with a few drops of 6 N H_2SO_4 to prevent precipitation. Stock solutions of mercuric chloride and lead nitrate were prepared in distilled water. All results are given in mg/l of Cu, Hg, and Pb. Solutions were delivered to the system *via* Marriotte bottles (Mount & Warner, 1965).

A composite 200 ml water sample for the estimation of each metal was collected from the four control tanks and from each group of the 20 tanks in which the mullets were exposed. Samples for copper were acidified and analysed for total copper using the method described by Arthur & Leonard (1970). Total mercury in the test water was analysed by the flameless atomic absorption technique (Kopp, Longbottom & Lobring, 1972; U.S. Environmental Protection Agency, 1972), and total lead by the Hach spectrophotometric method (Sandell, 1965). Each test concentration varied by not more than 1 mg/l from its nominal value.

All determinations of mortality or moribundity of the fish tested were made visually after 96 h exposure. The criterion for the death was set at the point where there was no opercular action and the fish could not be stirred to movement on removal from the tank. The condition and behaviour of fish were also recorded in detail. All fish that died were removed immediately from the tank, and length, weight, and sex noted; in case of survivors, symptoms of poisoning were also recorded. The measure of death and moribundity used was the calculated 96 h 50% lethal concentration (96-h LC_{50}). The values of LC_{50} and associated 95% fiducial limits were found by probit analysis when possible (Finney, 1971). For assays with insufficient data for probit analysis, LC_{50} values were determined by the methods of Spearman-Karber (Dondoroff *et al.*, 1951).

Blood sampling was made on a minimum of two to six representative fish in each concentration replicate. The fish was dried with absorbent tissue and fresh blood obtained by transection of the caudal area posterior to the anal fin. The drop of blood collected with a heparinized capillary tube was transferred to a glass slide. A second glass slide was used to make the smear by the slide technique. Four air-dried films from each fish were stained with a commercial preparation of May-Grunwald – (M.G.) Giemsa for 5 sec, followed immediately by immersion in distilled water for 10 sec. A differential count was derived from the first 100 cell types observed (lymphocytes, thrombocytes, and eosinophils) of the best three stained blood films (Gardner & Yevich, 1969). All equipment coming into contact with the blood was cooled on ice prior to use. Little or no haemolysis was observed. All plasma samples were immediately frozen in capped polyethylene 400 ml test tubes. Immediately prior to analysis, the samples were thawed and inspected for

fibrin strands; these were removed if present. Red blood cell counts were made according to Todd, Sanford & Wells (1953) and haematocrit values according to Hessor (1960), using 75 × 1.3 mm i.d. tubes. The cyanmethaemoglobin method was used to measure haemoglobin (Frankel & Reitman, 1963). An analysis of variance was used to determine the statistical significance of the difference between values for exposed fish and controls (Steel & Torrie, 1960). Confidence limits were set at 95%.

RESULTS

ACUTE TOXICITY STUDIES

The LC_{50} estimates after 96-h exposures to the three trace elements are given in Table I. It is evident that to fishes of nearly the same weight and length and

TABLE I

LC_{50} estimates of trace elements for *Liza macrolepis* after 96-h exposures.

Metal	Mercury	Lead	Copper
Toxicant	Mercuric chloride	Lead nitrate	Copper sulphate
Exp.	III	VI	X
Date, 1977	4th–8th May	11th–15th May	4th–8th June
Doses (mg l)	0.04–0.59	1.15–18.36	0.11–1.80
No. of fish exposed	120	120	120
Average length (cm)	6.6	6.8	6.2
Average weight (g)	3.8	4.1	3.0
Initial mortality	2nd day	2nd day	4th day
LC_{50}	0.38	14.61	1.43

therefore, presumably the same age, mercury is highly toxic; its LC_{50} is about one half that of copper, whereas lead requires a tenfold increase in concentration compared with copper to induce the same percentage of mortality. Symptoms of poisoning observed during the study were characteristic for each of the three metals. In fish exposed to toxic doses of mercury, the first noticeable symptoms were a general darkening of body coloration accompanied by the formation of vertical bars. For copper, symptoms started by the fish rising towards the surface of water, followed by a period of hyperactivity characterized by rapid pectoral and opercular action, erratic swimming or darting, and a gradual loss of equilibrium. The only obvious symptom for lead poisoning was darkening of the coloration.

HAEMATOLOGICAL INVESTIGATION

Normal blood picture of Liza macrolepis

The typical mature thrombocyte of *L. macrolepis* ranges in size from 22 to 30 μm, *i.e.*, three or four times that of a mature red cell of humans. It is ovoid in shape, with a regular outline. The cytoplasm occupies 10–15% of the total cell area, stains

TABLE II

Blood changes in *Liza macrolepis* surviving after 96-h exposures to various concentrations of mercury, lead, and copper.

Serial no.	Trace element	Dose mg/l	No. sampled	Red blood cells		Leucocytes		Hb g/100 ml	Haematocrit %
				$\times 10^6$/mm^3	%	% Lympho-cytes	% Eosino-phils		
1	Hg	Control	15	1.25 ± 0.52	90.80	4.31	4.89	6.3 ± 3.3	41.8
2		0.0368	10	1.09 ± 0.89	89.90	5.50	4.60	6.2 ± 2.0	43.5
3		0.0737	10	1.10 ± 0.48	90.09	6.12	3.79	5.8 ± 3.2	44.9
4		0.1475	10	0.76 ± 0.71	71.60	5.09	23.31	6.1 ± 3.1	39.8
5		0.2950	6	0.70 ± 0.26	72.68	4.68	22.64	4.2 ± 3.0	37.5
6		0.5900	4	0.68 ± 0.30	70.72	4.22	25.06	3.9 ± 2.9	33.4
7	Pb	Control	15	0.98 ± 0.79	87.58	5.01	7.41	5.9 ± 3.7	44.0
8		1.1475	10	1.26 ± 0.99	90.42	4.93	4.65	6.0 ± 3.4	39.3
9		2.2950	10	1.10 ± 0.08	90.07	2.47	7.46	5.9 ± 2.1	40.9
10		4.5900	10	0.78 ± 0.06	73.27	1.07	25.66	5.1 ± 3.6	42.0
11		9.1800	8	0.60 ± 0.13	69.36	1.24	29.40	4.6 ± 2.7	38.7
12		18.3600	2	0.62 ± 0.33	71.03	0.93	28.04	3.0 ± 1.8	36.6
13	Cu	Control	15	1.41 ± 0.66	92.78	3.63	3.59	6.2 ± 3.3	45.7
14		0.1125	10	1.14 ± 0.97	90.60	4.06	5.34	5.3 ± 4.5	35.1
15		0.2250	10	1.03 ± 0.92	89.04	3.85	7.11	6.0 ± 3.2	46.7
16		0.4500	10	0.89 ± 0.42	79.45	3.24	17.31	6.4 ± 2.4	37.2
17		0.9000	6	0.90 ± 0.21	80.34	2.66	17.00	5.6 ± 1.6	37.1
18		1.8000	4	0.87 ± 0.29	77.76	3.05	19.19	6.5 ± 1.5	41.3

slightly acidophilic with May-Grunwald stain, often with purple inclusion granules. The oval to round nucleus is usually eccentrically placed, occupying 85–90% of the cell, is pyknotic in character, and stains uniformly.

The usual appearance of eosinophils is characterized by cells in the size range of 28–35 μm, *i.e.*, four to five times the size of a mature human red blood cell. They show regular outlines and the cytoplasm occupies a total area between 60 and 65% of the cell. In the cell, the granules appear as large, spherical orange-brown bodies, more numerous than in thrombocytes, and often mixed with neutrophilic granules. The nucleus is a large round structure always eccentrically placed. The chromatin appears irregularly dense. There is sharp segregation of chromatin and parachromatin, the former being distributed in a linear arrangement of coarse strands. The nuclear membrane is delicate but clear. Normally, one to four nucleoli are present, but are often difficult to see.

The general size and shape of lymphocytes are similar to those of mammals, but the cells are larger and differ in their nuclear characteristics. The nucleus is relatively smaller and the staining affinities are more pronounced. The blood of *L. macrolepis* not only shows its peculiarities in the shape, size, and staining affinities, but is also characterized by specific red blood cell counts, haemoglobin content, and haematocrit values.

As shown in Table II, the number of red blood corpuscles varied from 0.98 to 1.41 $\times 10^6$/mm^3 in mullets not exposed to the trace elements. Although the haematocrit value of blood of Kuwait mullets, averaging 44%, appears high, it is within the expected limits of haematocrit values obtained in other studies of short duration. Slight, non-significant variations noted in haematocrit reading may be roughly correlated with erythrocytic count.

HAEMATOLOGICAL CHANGES INDUCED BY TRACE ELEMENTS

Although elliptocytosis is a common feature of mullet red cells, the morphological anomalies encountered in blood smears of exposed fish could not be classified using the terminology applicable to other animals. In some affected smears, the nucleus of the eosinophil cell was found to be irregularly divided into lobes, to assume an elongated spindle shape or to be joined together with broad bands of nuclear material. Calculating an index based upon the principle of Arneth-Cooke Count (Britton, 1963), would clearly indicate a shift to the left. Heavy vacuolization of the cytoplasm was noted in all cells with deviated nuclei.

Signs of aberrance in the granulocytes were characterized by deformed nuclei (oval-shaped) and a general reduction in granular mass. Although fishes maintained a relatively constant number of thrombocytes, they varied considerably in their other haematological responses to minimal concentrations of trace elements; even more, this variation in response was noted between individuals of exactly the same weight and length and under the same experimental conditions.

Approximately +1 anisocytosis was detected in experiments with lead at 4.59–

18.36 mg/l when 15–20% of the cells varied in size. Alterations in cell shape were only detectable at lethal doses (14.61–18.36 mg/l) where atypical nucleated drepanocyte-like cells (sickle cells) were a constant feature of the smears examined. Frequently, polychromasia was also identified in some of these smears. At 2.29 mg/l and 96-h exposure lead induced a significant change in thrombocyte structure. The nuclei appeared slightly altered, as indicated by their scalloped appearance. Exposed to minimal doses of lead (1.15–2.29 mg/l) the mean number of erythrocytes of some fish increased markedly, while that of others did not change. The variation among individuals was reflected in the ranges of the control and experimental groups. At 4.59 mg lead/l the mean number of erythrocytes decreased slightly; these changes continued upon exposure to 9.18 mg/l for 96 h, at which level the decrease reached the maximum of the three metals. The responses at 18.36 mg lead/l were similar to those of the preceding dose. Haemoglobin content was rarely affected by lead and only slight deviation was noted with the maximum dose (18.36 mg/l).

The effect of up to 9.18 mg Pb/l was to steadily increase the abundance of eosinophilic cells from 7.4% in the controls to an average of 29.4% in the toxic solution; lymphocytes showed a corresponding decrease.

Acanthrocytes started to appear in the blood of *L. macrolepis* at lethal doses (0.9 mg/l) of copper. The usual morphological structure of the erythrocyte nuclei was obscure in the affected cells; there were no discernible alterations to the cytoplasm. Eosinophilic number was the best indicator of stress under the influence of copper but was not as reliable for mercury, the effect of which was seen at lower concentrations. Increases in eosinophilic numbers were proportional to the concentrations of the trace element above 0.225 mg Cu/l. There was an increase in eosinophilic granulocytes at 0.113 mg/l, but the values did not significantly differ from those of controls. At 0.11 and 0.90 mg Cu/l, the mean number of lymphocytes and erythrocytes remained within physiological limits. Slight non-significant decreases were only noted at higher concentrations.

There were no discernible pathological formations of lymphocytes or erythrocytes under the effect of mercury in amounts from 0.037–0.59 mg/l. Moreover, the lowest concentration of mercury added to sea water that caused a significant change in blood of fish was 0.15 mg/l and brought about significant ($P < 0.05$) decreases in the number of erythrocytes but without any marked change in their form. The number of eosinophils consequently increased. Examination of blood showed a rapid and striking change among the cells of the eosinophil lineage after exposure to 0.15 mg Hg/l. 96 h after 0.59 mg Hg/l, the percentage of aberrated granulocytes reached a maximum. A comparison of changes in blood at 0.15, 0.29, and 0.59 mg Hg/l, showed that the magnitude of such changes appears to be roughly proportional to the concentration of the toxicant. Except for the maximum doses of mercury (0.29 and 0.59 mg/l) the haemoglobin content of blood rarely changed. Only a small number of samples was analysed and this together with the limitations of the method used makes any generalizations impossible.

Discussion

The measure of specific physiological and biochemical changes in the blood of fish exposed for short periods to potentially lethal environmental stress may provide a sensitive method for predicting the effects of sublethal exposures on survival, reproduction, and growth. This would allow a relatively rapid evaluation of the chronic toxicities of these compounds and help in diagnosis and better understanding of their effects in the biological systems. Physiological changes in the blood and tissue of fish exposed to varying degrees and types of environmental stress have been measured by several people. As early as 1926, Hall, Gray & Lepkovsky (1926) studied the influence of asphyxiation on the blood constituents of some marine fishes. Dawson (1935), Abegg (1950), Schiffman & Fromm (1959), and Jackim, Hamlin & Sonis (1970) investigated the effects of metals or drugs on the haemopoietic system of fish. The fact that these investigators worked with various species of fish, applied different techniques, and used different ways for measuring response, makes a general interpretation impossible

Some physical effects of pollution have been given by Higginbotham & Meyer (1950), Fujiya (1964), Lloyed & White (1967), and Bouck & Ball (1966, 1968), but the short term effects at lethal levels have not been correlated with diagnostic utility nor compared with changes in higher mammals.

Special attention was given to mercury, lead, and copper because of their toxicity and widespread distribution in the aquatic environment (Jensen & Jernelov, 1969; D'Itri, 1971; Committee on Biologic Effects of Atmospheric Pollutants, 1972; Gillespie, 1972; Peakall & Lovett, 1972). Degenerative changes in the cellular morphology of human peripheral blood, in association with known toxaemias, provided a basis for diagnostic application (Ponder & Ponder, 1942; Harper, 1968). In comparison, a survey of the literature shows a lack of correlated information on similar degenerative cytological processes in fish submitted to toxic products.

Our values for the blood factors as RBC count and haematocrit in the control fish are similar to those found by Katz (1951), Phillips (1958), and Barnhart (1969). The wide range of these may be explained by changes due to sex, physiological condition, handling or other variables acting within the groups of control (Snieszko, 1961; Hunn, Schoettger & Whealdon, 1968; Barnhart, 1969). The decrease in erythrocyte number indicates haemopathology familiar to lead poisoning in higher vertebrates, as well as other species of fish (Hardy *et al.*, 1973), where acute haemolytic crisis sometimes results in severe anaemia. Although the granulocytes appear homologous in a variety of vertebrates, the morphological characteristics in mullets may conceal possible differences in functional activities. Fey (1966), on the basis of electron microscope studies, considered the granulocytes of 25 different lower vertebrates, including teleosts, to be morphologically homologous to those of mammals.

The existence of similarities between teleostean and mammalian eosinophils

would appear to be strengthened by the observations of Catton (1951) who studied the structure and habit of these cells. He observed morphological similarities, but showed differences in the physiological activity of granular cells from teleost in comparison with those of mammalian origin. Recent studies on the fine structure of the teleost blood cells (Weinreb, 1963) support these similarities. From his studies with fishes and based on granular size, Jordan (1938) concluded that two different teleostean eosinophils exist, namely renal and intestinal mucosa. The marked eosinophilia associated with mercury and copper poisoning in mullet fingerlings, however, is reminiscent of a similar response in humans suffering from arsenic or phosphorus poisoning (Ringeon, 1938; Harrison, 1962).

Although the lymphocytes of teleosts attracted the attention of early observers (Drury, 1915; Jordan, 1938), the knowledge of the rôle of these cells in fish remains limited. Little, if any, research has been done, therefore, on the functional significance of lymphocytopenia noted in experiments with lead. On the contrary, the eosinophils of some teleosts, and those of mammalian origin, do show similarities since in most instances these cells are known to respond to parasitism, exhibit phagocytic properties, and are active participants during inflammatory reactions (Ringoen, 1938; Duthie, 1939; Sturgis, 1955; Litt, 1964; Reichenbach-Klinke, 1966; Patt & Patt, 1969; Gardner & Yevich, 1970). Since eosinophilia in mammals is apparently a reaction against toxic products of autogenous or foreign protein, eosinophilia in mullets could possibly represent a chemotactic response linked to increased renal uptake of metal.

References

Aaronson, R. M. & H. M. Spiro, 1973. Mercury and the gut. *Am. J. Dig. Dis.*, Vol. 18, pp. 583–594.

Abegg, R., 1950. Some effects of inorganic salts on the blood specific gravity and tissue fluids of the bluegill, *Lepomis mactochirus* (Raf). *Physiol. Zool.*, Vol. 23, pp. 124–134.

Arthur, J. W. & E. N. Leonard, 1970. Effects of copper on *Gammarus pseudolimnaeus*, *Physa integra* and *Campeloma decisum* in soft water. *J. Fish. Res. Bd Can.*, Vol. 27, pp. 1277–1283.

Barnhart, R. A., 1969. Effects of certain variables on hematological characteristics of rainbow trout. *Trans. Am. Fish. Soc.*, Vol. 98, pp. 411–418.

Bouck, G. R. & R. C. Ball, 1966. Influence of capture methods on blood characteristics and mortality in rainbow trout, (*Salmo gardneri*). *Trans. Am. Fish. Soc.*, Vol. 95, pp. 170–176.

Bouck, G. R. & R. C. Ball, 1968. Comparative electrophoretic patterns of lactate dehydrogenase in three species of trout. *J. Fish. Res. Bd Can.*, Vol. 25, pp. 1323–1331.

Britton, C. J. C., 1963. *Disorders of the blood*. Grune & Stratton, New York, 9th edition, 578 pp.

Burdick, G. E., 1966. Use of bioassay in determining levels of toxic wastes harmful to aquatic organisms. Symposium on water quality criteria to protect aquatic life. *Am. Fish. Soc., Spec. Publ.*, No. 16, Vol. 4, pp. 7–11.

Catton, W. T., 1951. Blood cell formation in certain teleost fishes. *Blood*, Vol. 6, pp. 39–60.

Committee on Biologic Effects of Atmospheric Pollutants, 1972. *Lead. Airborne lead in perspective*. National Academy of Sciences, Washington, D.C., 121 pp.

Dawson, A. B., 1935. The hemopoietic response in the catfish, *Ameiurus nebulosus*, to chronic lead poisoning. *Biol. Bull. mar. biol. Lab., Woods Hole*, Vol. 68, pp. 335–346.

D'Itri, F. M., 1971. *Mercury in the aquatic ecocystem*. Inst. of Water Res. Michigan State University, Lansig, Michigan, 87 pp.

Dondoroff, P., B. G. Andersen, G. E. Burdick, P. S. Galsoff, W. B. Hart, R. Patrick, E. R.

STRONG, E. W. SURBER and W. M. VAN HORN, 1951. Bioassay methods for the evaluation of acute toxicity of industrial wastes to fish. *Sewage ind. Wastes*, Vol. 22, pp. 1380–1397.

DRURY, A. N., 1915. The eosinophil cell of teleostean fish. *J. Physiol.*, Vol. 49, pp. 349–366.

DUTHIE, E. S., 1939. The origin, development and function of blood cells in certain marine teleosts. Part I. Morphology. *J. Anat.*, Vol. 73, pp. 396–412.

FEY, F., 1966. Vergleichende Hamozytologie nieder Vertebraten III. Granlozyten. *Folio Haemet.*, Bd 86, S. 1–20.

FINNEY, D. J., 1971. *Probit analysis*. Cambridge University Press, Cambridge, 3rd edition, 333 pp.

FRANKEL, S. & S. REITMAN, 1963. Editors. *Gradwohl's clinical laboratory methods and diagnosis*. C. V. Mosby and Co., St. Louis, Missouri, 6th edition, Vol. I, 125 pp., Vol. II, 1113 pp.

FROLOVA, L. K., 1960. The effect of inorganic cobalt on the morphological picture of the blood of *Cyprinus carpio*. *Dokl. Akad. Nauk SSSR.*, Vol. 131, pp. 983–984.

FUJIYA, M., 1964. Physical estimation on the effects of pollutants upon aquatic organisms. *2nd Int. Conf. Water Pollut. Res., Sect. III*, Vol. 15, pp. 1–6.

GARDNER, G. R. & P. P. YEVICH, 1969. Studies on the blood morphology of three estuarine cyprinodontform fishes. *J. Fish. Res. Bd Can.*, Vol. 26, pp. 433–447.

GARDNER, G. R. & P. P. YEVICH, 1970. Histological and hematological responses of an estuarine teleost to cadmium. *J. Fish. Res. Bd Can.*, Vol. 27, pp. 2185–2196.

GILLESPIE, D. C., 1972. Mobilization of mercury from sediments into guppies, (*Paecilia reticulata*). *J. Fish. Res. Bd Can.*, Vol. 29, pp. 1035–1041.

HALL, G. F., I. E. GRAY & S. LEPKOVSKY, 1926. The influence of asphyxiation on the blood constituents of marine fishes. *J. biol. Chem.*, Vol. 64, pp. 549–555.

HAMMOND, P. B., 1969. Lead poisoning. An old problem with a new dimension. In, *Essays in toxicology*, Vol. I, edited by F. R. Blood, Academic Press Inc., New York, pp. 116–155.

HARDY, H. L., R. I. CHAMBERLIN, C. C. MALOOF, C. W. BOYLEN Jr & M. C. HOWELL, 1973. Lead as an environmental poison. *Clin. Pharmac. Ther.*, Vol. 12, pp. 982–1002.

HARPER, T. A., 1968. *The peripheral blood film*. Butterworth & Co. Ltd, London, England, 134 pp.

HARRISON, T. R., 1962. Editor. *Principles of internal medicine*. McGraw Hill Book Company, Inc., New York, N.Y., 4th edition, pp. 101–123.

HESSOR, E. F., 1960. Methods for routine fish hematology. *Progve Fish. Cult.*, Vol. 22, pp. 164–171.

HIGGINBOTHAM, A. C. & D. K. MEYER, 1950. Determination of the physical condition of fish. I. Some blood analyses of southern channel catfish. *J. Fla Acad. Sci.*, Vol. 2, pp. 119–125.

HOUSTON, A. H. & M. A. DE WILDE, 1968. Hematological correlations in the rainbow trout, *Salmo gairdneri*. *J. Fish. Res. Bd Can.*, Vol. 25, pp. 173–176.

HUBSCHMAN, J. H., 1967. Effects of copper on crayfish, *Orconectes rusticus* (*Girard*). I. Acute toxicity. *Crustaceana*, Vol. 12, pp. 33–42.

HUNN, J. B., R. A. SCHOETTGER & E. W. WHEALDON, 1968. Observations on the handling and maintenance of bioassay fish. *Progve Fish. Cult.*, Vol. 30, pp. 164–167.

JACKIM, E., J. M. HAMLIN & S. SONIS, 1970. Effects of metal poisoning of five liver enzymes in the killifish (*Fundulus hetroclitus*). *J. Fish Res. Bd Can.*, Vol. 27, pp. 383–390.

JENSEN, S. & A. JERNELOW, 1969. Biological methylation of mercury in aquatic organisms. *Nature Lond.*, Vol. 223, pp. 753–754.

JORDAN, H. E., 1938. Comparative hematology. In, *Handbook of hematology*, Vol. 2, edited by H. Downey, Paul B. Hoeber, Inc., New York, U.S.A., pp. 699–862.

KATZ, M., 1951. The number of erythrocytes in the blood of the silver salmon. *Trans. Am. Fish. Soc.*, Vol. 80, pp. 184–193.

KOPP, J. F., M. C. LONGBOTTOM & L. B. LOBRING, 1972. Cold vapor method for detecting mercury. *J. Am. Wat. Wks Ass.*, Vol. 64, pp. 20–25.

KURONUMA, B. & Y. ABE, 1972. *Fishes of Kuwait*. Kuwait Institute for Scientific Research, Kuwait, 123 pp.

LICHATOWICH, J. A., P. W. O'KEEFE, J. A. STRAND & W. L. TEMPLETON, 1973. Development of methodology and apparatus for the bioassay of oil. *Proceedings joint conference on prevention and control of spills*, March 13–15, 1973, Washington D.C., American Petroleum Inst., U.S. Coast Guard and Environmental Protection Agency, pp. 659–666.

LITT, M., 1964. Eosinophils and antigen–antibody reactions. *Am. N.Y. Acad. Sci.*, Vol. 116, pp. 964–985.

LLOYED, R. & W.R. WHITE, 1967. Effect of high concentration of carbon dioxide on the ionic composition of rainbow trout blood. *Nature, Lond.*, Vol. 216, pp. 1341–1342.

MILLER, M.W. & T.W. CLARKSON, 1973. Editors. *Mercury, mercurials and mercaptans*. Charles C. Thomas, Publ. Springfield, Illinois, U.S.A., 111 pp.

MOUNT, D.I. & W.A. BRUNGS, 1967. A simplified dosing apparatus for fish toxicology studies. *Water Res.*, Vol. 1, pp. 21–29.

MOUNT, D.I. & R.E. WARNER, 1965. *A serial dilution apparatus for continuous delivery of various concentrations of materials in water*. U.S. Public Health Service Publ. No. 999-WP-23, 16 pp.

MOUNT, D.I. & C.E. STEPHAN, 1967. A method for detecting cadmium poisoning in fish. *J. Wildl. Mgmt.*, Vol. 31, pp. 168–172.

Occupational Health and Industrial Pollution Control Section – Ministry of Public Health, 1976. *Annual Report*, Kuwait, 1976, 20 pp.

PATT, D.I. & G.R. PATT, 1969. *Comparative vertebrate histology*. Harper and Row Publ., New York, N.Y. & Evanston, Illinois, U.S.A., 438 pp.

PEAKALL, D.B. & R.J. LOVETT, 1972. Mercury, its occurrence and effects in the ecosystem. *Biosciences*, Vol. 22, pp. 20–25.

PHILLIPS Jr, A.M., 1958. The organic composition of brook and brown trout blood. *Progve Fish. cult.*, Vol. 20, pp. 114–116.

PONDER, E. & R.O. PONDER, 1942. The cytology of polymorphnuclear leucocyte in toxic conditions. *J. Lab. clin. Med.*, Vol. 28, pp. 316–322.

REICHENBACH-KLINKE, H.H., 1966. The blood components of fish with relation to parasites, infection and water pollution. *Bull. Off. int. Epizool.*, Vol. 65, pp. 1039–1054.

RINGOEN, A.R., 1938. Eosinophil leucocytes and eosinophilia. In. *Handbook of hematology*, edited by H. Downey, Paul B. Hoeber, Inc., New York, pp. 179–229.

SANDELL, E.B., 1965. *Colorimetric determination of traces of metals*. Interscience Publ., Inc., New York, 3rd edition, 1032 pp.

SCHIEFMAN, R.A. & P.O. FROMM, 1969. Chromium induced changes in the blood of rainbow trout, *Salmo gairdneri*. *Sewage Ind. Wastes*, Vol. 31, pp. 205–211.

SNIESZKO, S.F., 1961. Microhematocrit values in rainbow trout, brown trout and brook trout. *Progve Fish. Cult.*, Vol. 23, pp. 114–119.

SPRAGUE, J.B., 1964. Avoidance of copper-zinc solutions by young salmon in the laboratory. *J. Wat. Pollut. Control Fed.*, Vol. 36, pp. 990–1004.

STEEL, R.G.D. & J.H. TORRIE, 1960. *Principles and procedures of statistics with special reference to the biological sciences*. McGraw Hill, Inc., New York, 481 pp.

STURGIS, C.C., 1955. *Hematology*. Charles C. Thomas Publ., Springfield, Illinois, U.S.A., 2nd edition, 1222 pp.

TODD, J.C., A.H. SANFORD & B.B. WELLS, 1953. *Clinical diagnosis by laboratory methods*. W.B. Saunders Co., Philadelphia, Pa., 12th edition, 988 pp.

U.S. Environmental Protection Agency, 1972. *Mercury in water-provisional method*. Analytical Quality Control Laboratory, Cincinnati, Ohio, 5 pp.

WEINREB, E.L., 1963. Studies on the fine structure of teleost blood cells. 1. Peripheral blood. *Anat. Rec.*, Vol. 147, pp. 219–238.